

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

1 (original). A process for determining the quantitative and qualitative profile of the repertoire of a given type of an immunoglobulin heavy chain expressed by a lymphocyte B population present in a tissue sample, characterized in that it comprises the following steps:

(a) obtaining either the cDNA from the mRNA expressed from the tissue sample or the cellular DNA extract of the tissue sample;

(b) performing the amplification of the cDNA obtained at the step (a) with a set of VH forward primers capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the variable segments (VH) of immunoglobulin heavy chains, said variable segments being distributed among VII subgroups, associated with a C11 reverse primer, or a mixture thereof, capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of a given type of an immunoglobulin heavy chain; and

(c) determining the quantitative and qualitative profile of the repertoire of said type of immunoglobulin heavy chain for each VH subgroup.

2 (original). The process for determining the quantitative and qualitative profile according to claim 1, characterized in that separated amplifications are performed for each of the VH subgroups.

3 (original). The process for determining the quantitative and qualitative profile according to claim 2, characterized in that the separated amplifications are real-time separated amplifications, said real-time amplifications being performed using a CH

labeled reverse probe, preferably a CH labeled reverse hydrolysis-probe, capable of specifically hybridizing in stringent conditions with the constant segment of the given type of immunoglobulin heavy chain and capable of emitting a detectable signal everytime each amplification cycle occurs, and characterized in that the signal obtained for each VH subgroup is measured.

4 (original). The process for determining the quantitative and qualitative profile according to claim 2 or 3, characterized in that the separated amplification products obtained for each of the VH subgroups are further elongated using a CH labeled reverse probe capable of specifically hybridizing in stringent conditions with the constant segment of the given type of immunoglobulin heavy chain and capable of emitting a detectable signal, and characterized in that the elongation products are separated, for each of the VH subgroups, relative to their length, the signal obtained for the separated elongation products is measured, and the quantitative and qualitative profile of the labeling intensity relative to the elongation product length is established, for each of the VH subgroups individually.

5 (Currently Amended). The process for determining the quantitative and qualitative profile according to ~~anyone of claims 1 to~~ claim 4, characterized in that the set of VH forward primers comprises at least the 8 following subgroups of VH primers corresponding to the VH subgroups:

- the VH1 primers having the sequences SEQ ID No. 1 to SEQ ID No. 3,
and
- the VH2 primer having the sequence SEQ ID No. 4, and

- the VH3a primers having the sequences SEQ ID No. 5 and SEQ ID No. 6, and
- the VH3b primers having the sequences SEQ ID No. 7 to SEQ ID No. 10, and
- the VH4 primers having the sequences SEQ ID No. 11 to SEQ ID No. 12, and
- the VH5 primers having the sequence SEQ ID No. 13, and
- the VH6 primer having the SEQ ID No. 14, and
- the VH7 primer having the SEQ ID No. 15.

6 (original). The process for determining the quantitative and qualitative profile according to claim 5, characterized in that the sequences SEQ ID No. 1 to SEQ ID No. 15 may contain at least one to three point mutations, except for the nucleotides 1 to 6 of their 3' part.

7 (Currently Amended). The process for determining the quantitative and qualitative profile according to claim 4 to 6, characterized in that the CH reverse primer is selected from the CH reverse primers capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the constant segments (CH) of the IgM heavy chain, the IgE heavy chain and the IgA heavy chain.

8 (original). The process for determining the quantitative and qualitative profile according to claim 7, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgM heavy chain, the CH reverse primer has the

sequence SEQ ID No. 26, or the sequence SEQ ID No. 26 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

9 (original). The process for determining the quantitative and qualitative profile according to claim 7, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgE heavy chain, the CH reverse primer has the sequence SEQ ID No. 33, or the sequences SEQ ID No. 33 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

10 (Currently Amended). The process for determining the quantitative and qualitative profile according to ~~any one of claim 4 to 6~~, characterized in that, when the given type of immunoglobulin heavy chain is the IgG type, a mixture of two CH reverse primers is associated with the set of VH forward primers, said two CH reverse primers having the sequences SEQ ID No. 27 and SEQ ID No. 28, or the sequences SEQ ID NO 27 and SEQ ID No. 27 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

11 (Currently Amended). The process for determining the quantitative and qualitative profile according to claim 7 ~~or 8~~, characterized in that, when the given type of immunoglobulin heavy chain is the IgM heavy chain and when the separated amplifications are real-time separated amplifications, the CH labeled hydrolysis-probe has the sequence SEQ ID No. 29, or the sequence SEQ ID No. 29 wherein at least one point mutation may occur.

12 (Currently Amended). The process for determining the quantitative and qualitative profile according to claim 7-~~or~~8, characterized in that, when the given type of immunoglobulin heavy chain is an IgM heavy chain and when the separated amplification products obtained for each of the VH subgroups are further elongated, the CH labeled reverse probe has the sequence SEQ ID No. 30, or the sequence SEQ ID No. 30 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

13 (Currently Amended). The process for determining the quantitative and qualitative profile according to claim 7-~~or~~9, characterized in that, when the given type of immunoglobulin heavy chain is an IgE heavy chain and when the separated amplifications are real-time separated amplifications, the CH labeled hydrolysis-probe has the sequence SEQ ID No. 36, or the sequence SEQ ID No. 36 wherein at least one point mutation may occur.

14 (Currently Amended). The process for determining the quantitative and qualitative profile according to claim 7-~~or~~9, characterized in that, when the given type of immunoglobulin heavy chain is an IgE heavy chain and when the separated amplification products obtained for each of the VH subgroups are further elongated, the CH labeled reverse probe has the sequence SEQ ID No. 37, or the sequence SEQ ID No. 37 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

15 (original). The process for determining the quantitative and qualitative profile according to claim 10, characterized in that, when the given type of immunoglobulin heavy chain is an IgG heavy chain and when the separated amplifications are real-time

separated amplifications, the CH labeled hydrolysis-probe has the sequence SEQ ID No. 34, or the sequence SEQ ID No. 34 wherein at least one point mutation may occur.

16 (original). The process for determining the quantitative and qualitative profile according to claim 10, characterized in that, when the given type of immunoglobulin heavy chain is an IgG heavy chain and when the separated amplification products obtained for each of the VH subgroups are further elongated, the CH labeled reverse probe has the sequence SEQ ID No. 35, or the sequence SEQ ID No. 35 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

17 (Currently Amended). The process for determining the quantitative and qualitative profile according to ~~claims 2 to~~ 16, characterized in that further separated amplifications for each of the JH subgroups are performed from the separated amplification products obtained for at least one given VH subgroup of the VH subgroups with the CH reverse primer,

said further separated amplifications being performed using a VH internal forward primer corresponding to the given VH subgroup, and associated with a set of JH reverse primers corresponding to the JH subgroups and capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the junction segments of the given type of immunoglobulin heavy chain.

18 (original). The process for determining the quantitative and qualitative profile according to claim 17, characterized in that the further separated amplifications are real-time amplifications performed using a VH labeled forward probe, preferably a VH labeled forward hydrolysis-probe, capable of specifically hybridizing in stringent conditions with the variable segment of the given type of immunoglobulin heavy chain

and capable of emitting a detectable signal every time each amplification cycle occurs, and characterized in that the signal obtained for each of the JH subgroups is measured.

19 (Currently Amended). The process for determining the quantitative and qualitative profile according to ~~claims 17 or~~ 18, characterized in that, when the given VII subgroup is the VH5 subgroup, the VH5 internal forward primer has the sequence SEQ ID No. 31, wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

20 (original). The process for determining the quantitative and qualitative profile according to claim 19, characterized in that the VII labeled forward hydrolysis-probe has the sequence SEQ ID No. 32, or the sequence SEQ ID No. 32 wherein at least one point mutations may occur.

21 (Currently Amended). The process for determining the quantitative and qualitative profile according to ~~any one of claims 2 to~~ 16, characterized in that separated elongations are performed for each of the JH subgroups from the separated amplification products obtained for at least one given VH subgroup of the VII subgroups with the CH primer,

said further separated elongations being performed using a set of JH labeled reverse primers corresponding to JH subgroups and capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the junction segments of the given type of immunoglobulin heavy chain, said JH labeled reverse primers being capable of emitting a detectable signal,

and characterized in that the elongation products are separated, for each of the JH subgroups, relative to their length, the signal obtained for the separated elongation products is measured, and the quantitative and qualitative profile of the labeling intensity relative to the elongation product length is established for each of the JH subgroups for the given VH subgroup.

22 (Currently Amended). The process for determining the quantitative and qualitative profile according to ~~anyone of claims 17 to 21~~, characterized in that the set of JH forward primers, optionally labeled, comprises at least the 6 following subgroups of JH primers corresponding to the JH subgroups:

- the JH 1 primers having the sequences SEQ ID No. 16, and
- the JH2 primer having the sequence SEQ ID No. 17, and
- the JH3 primer having the sequences SEQ ID No. 18, and
- the JH4 primers having the sequences SEQ ID No. 19 to SEQ ID No. 20,

and

- the JH5 primers having the sequence SEQ ID No. 22, and
- the JH6 primer having the SEQ ID No. 23 to SEQ ID No. 25.

23 (original). The process for determining the quantitative and qualitative profile according to claim 5, characterized in that the sequences SEQ ID No. 1 to SEQ ID No. 15 may contain at least one to three point mutations, except for the nucleotides 1 to 6 of their 3' part.

24 (Withdrawn). A set of VH forward primers capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the variable segments (VH) of immunoglobulin heavy chains, said variable segments being distributed among

at least 8 VH subgroups, associated with a CH reverse primer, or a mixture thereof, capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of a given type of an immunoglobulin heavy chain, characterized in that the set of VH forward primers comprises at least the 8 following subgroups VII primers corresponding to the VH subgroups:

- the VH1 primers having the sequences SEQ ID No. 1 to SEQ ID No. 3, and
- the VH2 primers having the sequence SEQ ID No. 4, and
- the VH3a primers having the sequences SEQ ID No. 5 and SEQ ID No. 6, and
- the VH3b primers having the sequences SEQ ID No. 7 and SEQ ID No. 10, and
- the VH4 primers having the sequences SEQ ID No. 11 and SEQ ID No. 12, and
- the VH5 primers having the sequence SEQ ID No. 13, and
- the VH6 primers having the sequence SEQ ID No. 14, and
- the VH7 primers having the sequence SEQ ID No. 15.

25 (Withdrawn). The set of VH forward primers according to claim 24, characterized in that the sequences SEQ ID No. 1 to SEQ ID No. 15 may contain at least one to three point mutations, except for the nucleotides 1 to 6 of their 3' part.

26 (Withdrawn). The set of VH forward primers according to claim 24 or 25, characterized in that the CH reverse primer is selected from the CH reverse primers capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the constant segments (CH) of the IgM heavy chain, the IgE heavy chain and the IgA heavy chain.

27 (Withdrawn). The set of VH forward primers according to claim 26, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgM heavy chain, the CH reverse primer has the sequence SEQ ID No. 26, or the sequence SEQ ID No. 26 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

28 (Withdrawn). The set of VH forward primers according to claim 26, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgE heavy chain, the CH reverse primer has the sequence SEQ ID No. 33, or the sequence SEQ ID No. 33 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

29 (Withdrawn). The set of VH forward primers according to claim 24 or 25, characterized in that, when the given type of immunoglobulin heavy chain is the IgG type, a mixture of two CH reverse primers is associated with the set of VH forward primers,

said two CH reverse primers having the sequences SEQ ID No. 27 and SEQ ID No. 28, or the sequences SEQ ID No. 27 and SEQ ID No. 28 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

30 (original). A method for the *in vitro* diagnosis of a condition associated with an abnormal expression of the repertoire of a given type an immunoglobulin heavy chain by a lymphocyte B population in a subject, characterized in that it comprises the following steps:

- (1) determining the quantitative and qualitative profile of the given type of immunoglobulin heavy chain from a tissue sample of said subject according to anyone of claims I to 23,
- (2) comparing the quantitative and qualitative profile obtained at the step (1) with a control quantitative and qualitative profile of said given type of immunoglobulin heavy chain, the demonstration of a significant modification of the profile obtained at the step (I) being significant of such a condition in the subject.

31 (original). The method for the *in vitro* diagnosis according to claim 30, characterized in that the condition is an auto-immune disease, a B cell lymphoma or an immunodepressive disease.

32 (original). The method for the *in vitro* diagnosis according to claim 30, characterized in that the condition results from a bone marrow transplantation, from a vaccinal test or from an allergic reaction.

33 (original). A method for the *in vitro* follow-up of a treatment of a condition associated with an abnormal expression of the repertoire of a given type of an immunoglobulin heavy chain by a lymphocyte B population in a subject, characterized in that it comprises the following steps:

- (1) optionally, determining before the treatment the quantitative and qualitative profile of the given type of immunoglobulin heavy chain from a tissue sample of said subject according to anyone of claims I to 19,

- (2) determining, during the treatment, the quantitative and qualitative profile of the given type of immunoglobulin heavy chain at given times from tissue samples of said subject according to anyone of claims 1 to 19,
- (3) comparing the quantitative and qualitative profiles obtained at the step (2) and optionally at the step (1) with each others and optionally with a control quantitative and qualitative profile of the given type of immunoglobulin heavy chain, the demonstration of a significant modification of the profile obtained at the step (1) being significant of such a condition in the subject.

34 (original). The method for the *in vitro* follow-up according to claim 33, characterized in that the condition is an auto-immune disease, a B cell lymphoma or an immunodepressive disease,

35 (original). The method for the *in vitro* follow-up according to claim 33, characterized in that the condition results from a bone marrow transplantation, from a vaccinal test or from an allergic reaction.

36 (Withdrawn). A kit for determining the quantitative and qualitative profile of the repertoire a given type of an immunoglobulin heavy chain expressed by a lymphocyte B population present in a tissue sample, characterized in that it comprises the set of VII forward primers according to anyone of claims 24 to 29 associated with the CH reverse primer.

37 (Withdrawn). The kit according to claim 36, characterized in that it further comprises a set of JH reverse primers, optionally labeled, corresponding to the JH subgroups and capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the junction segments of the given type of immunoglobulin heavy chain.

38 (Withdrawn). The kit according to claim 37, characterized in that the set of JH reverse primers comprises the 6 following subgroups of JH primers corresponding to the JH subgroups:

- the JH1 primer having the sequence SEQ ID No. 16, and
 - the JH2 primer having the sequence SEQ ID No. 17, and
 - the JH3 primer having the sequence SEQ ID No. 18, and
 - the JH4 primers having the sequences SEQ ID No. 19 to SEQ ID No. 21,
- and
- the JH5 primer having the sequence SEQ ID No. 22, and
 - the JH6 primers having the sequences SEQ ID No. 23 to SEQ ID No. 25.

39 (original). The kit according to claim 38, characterized in that the sequences SEQ ID No. 16 to SEQ ID No. 25 may contain at least one to three point mutations, except for the nucleotides 1 to 6 of their 3' part.

40 (Withdrawn). Use of the kit according to anyone of claims 36 to 39, *in vitro* diagnosis of a condition associated with an abnormal expression of the repertoire of a given type of an immunoglobulin heavy chain by a lymphocyte B population in a subject.

41 (Withdrawn). The use of the kit according to claim 41, characterized in that the condition is an auto-immune disease, a B cell lymphoma or an immunodepressive disease.

42 (Withdrawn). The use of the kit according to claim 41, characterized in that the condition results from a bone marrow transplantation, from a vaccinal test or from an allergic reaction.

43 (New). The process for determining the quantitative and qualitative profile according to claim 1, wherein VH forward primers comprise SEQ ID NO: 11 or SEQ ID NO: 13.

44 (New). The process for determining the quantitative and qualitative profile according to claim 1, wherein JH forward primers comprise SEQ ID NO: 16 or SEQ ID NO: 17.

45 (New). The process for determining the quantitative and qualitative profile according to claim 1, wherein CH forward primers comprise SEQ ID NO: 40 or SEQ ID NO: 41.